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“Bath Salts” and Other Designer Drugs

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Targeted LC-QQQ MS Screening of Cathinone Derivatives and Other Designer Drugs in Serum

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Designer Drugs

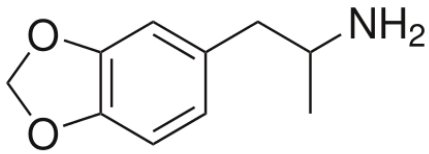
Designer Drugs

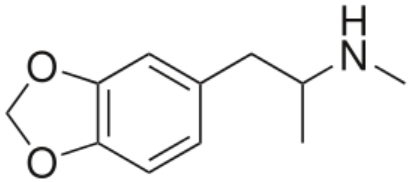
- “Drugs that are created (or marketed, if they had already existed) so as to avoid the provisions of existing drug laws, usually by preparing analogs or derivatives of existing drugs by modifying their chemical structure to varying degrees, or less commonly by finding drugs with entirely different chemical structures that produce similar subjective effects to illegal recreational drugs.”

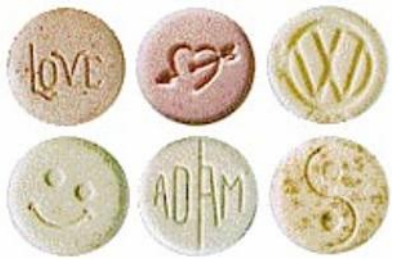
- See www.erowid.org and PiHKAL (“*Phenethylamines I Have Known and Loved*”) and TiHKAL (“*Tryptamines I Have Known and Loved*”) books by Dr. Alexander Shulgin.

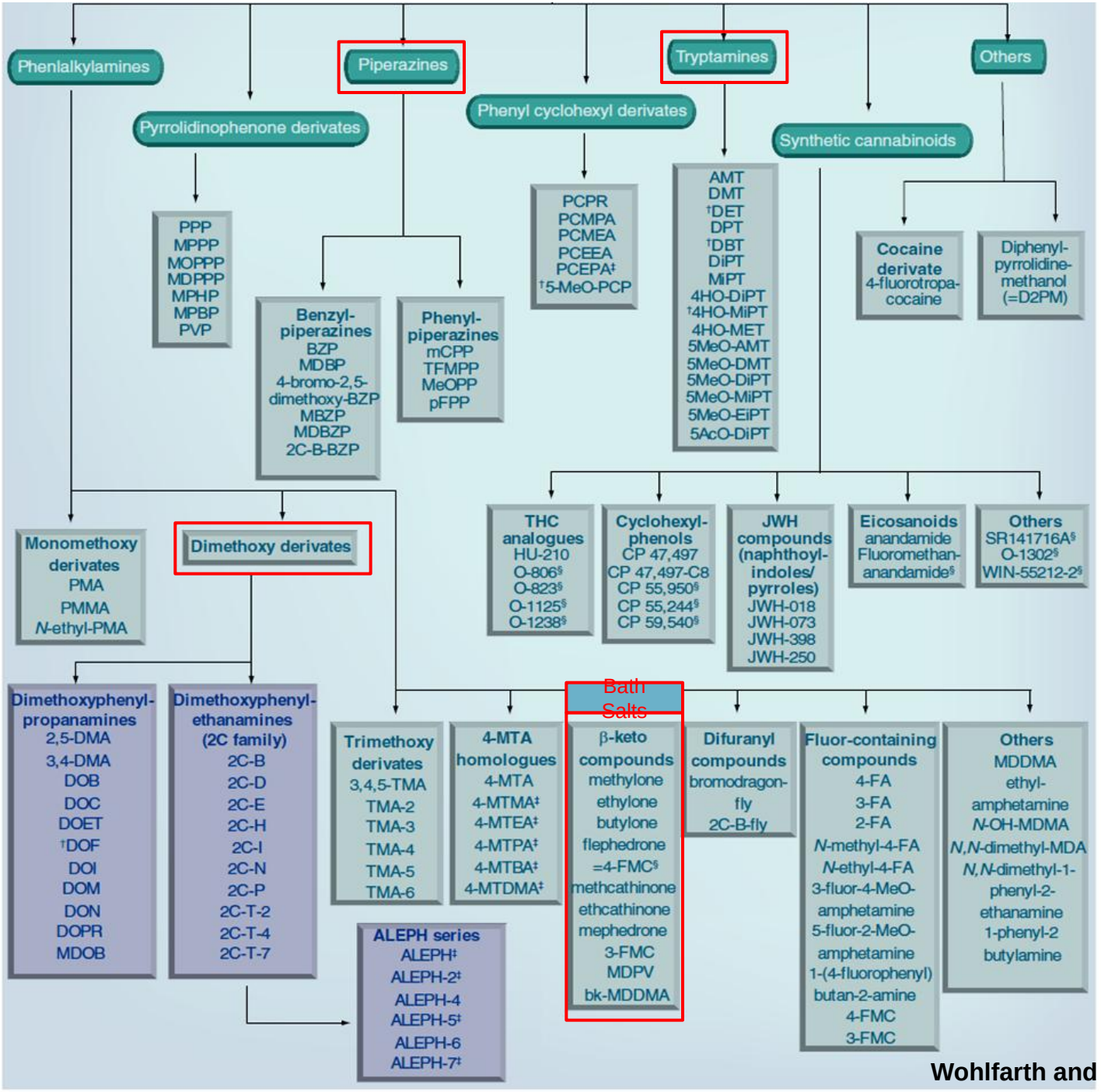
MDA and MDMA - The First Designer Drugs?

- MDA, the drug originally known as "Ecstasy," was first synthesized in 1910.
- Used variously as an antitussive, an ataractic, and as an anorexiant.
- MDMA was first synthesized by Merck in 1914 as an appetite suppressant but never marketed as such.
- Now popular as illegally synthesized drugs.



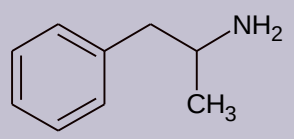
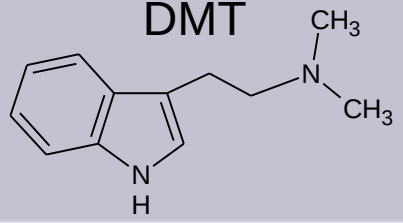
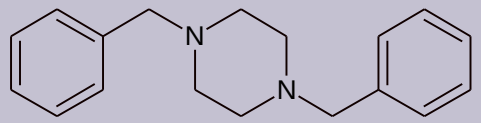
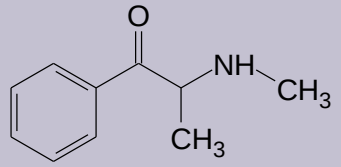
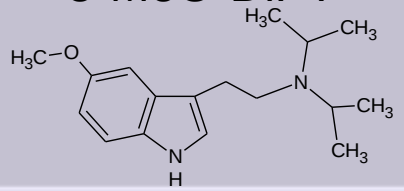
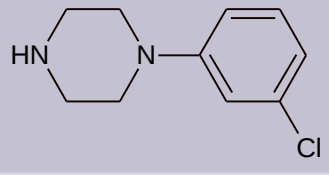
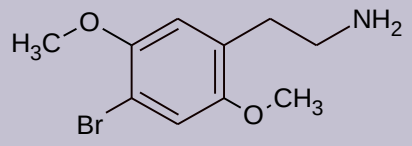
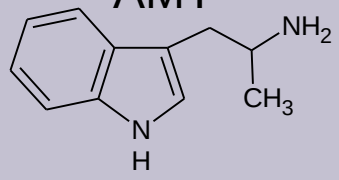
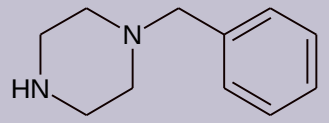




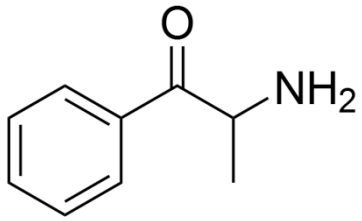


Wohlfarth and Weinmann, 2010.

Examples of Designer Drugs

Phenethylamines	Tryptamines	Piperazines
Amphetamine 	DMT 	DBZP 
Methcathinone 	5-MeO-DiPT 	mCPP 
2C-B 	AMT 	BZP 

Synthetic Cathinones ("Bath Salts")



- Cathinone is a CNS stimulant found in the leaves of the "khat" bush (*Catha edulis*). Khat is Ethiopia's fourth biggest export and is growing every year.
- Since the mid-2000s, unregulated cathinone derivatives have appeared in the European and American recreational drug market.
- Most commonly available derivatives are 3,4-methylenedioxypyrovalerone (MDPV), mephedrone ("meow-meow"), and methylone.
- Banned by DEA under emergency action on October 21, 2011.

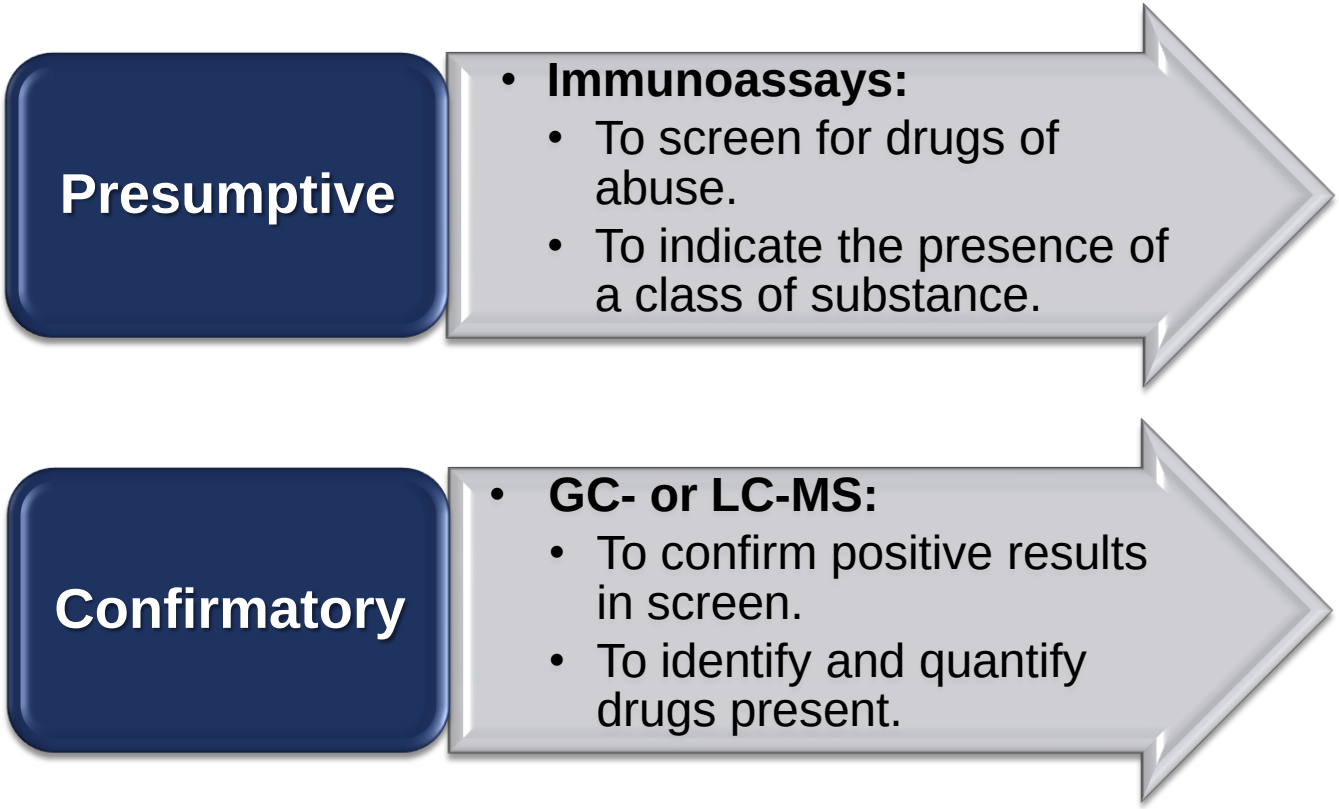
Physiological and Psychological Effects

- Aggression.
- Agitation.
- Breathing difficulty.
- Bruxism.
- Confusion.
- Dizziness.
- Extreme anxiety, sometimes progressing to violent behavior.
- Fits and delusions.
- Hallucinations.
- Headache.
- Hypertension.
- Increased body temperature, chills, sweating.
- Insomnia.
- Kidney pain.
- Lack of appetite.
- Liver failure.
- Loss of bowel control.
- Muscle spasms.
- Muscle tenseness.
- Nausea, stomach cramps, and digestive problems.
- Nosebleeds.
- Psychotic delusions.
- Pupil dilation.
- Renal failure.
- Rhabdomyolysis.
- Severe paranoia.
- Suicidal thoughts.
- Tachycardia.
- Tinnitus.
- Vasoconstriction.

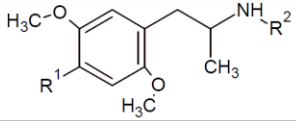
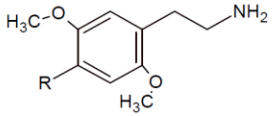
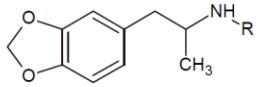
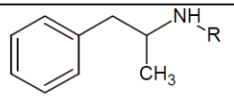
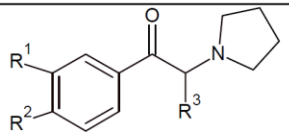
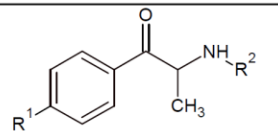
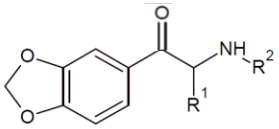
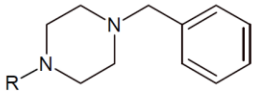
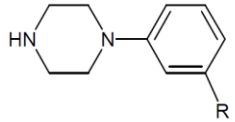
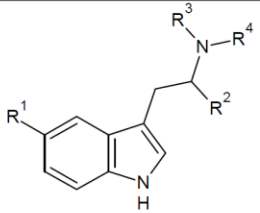
Forensic Toxicology Issues for Designer Drugs

- Possible high potency.
- Fatal overdose does occur.
- Lack of available information on clinical action, toxicokinetics, lethal levels.
- Variable legal status.
- Unknown or new entities.
- Cross-reactivity (or lack thereof) in immunoassays.
- Lack of comprehensive analytical methods for screening, confirmation and/or quantification.

Toxicological Analysis of Designer Drugs



- Goal: Develop LC-QQQ-MS screening/confirmatory analytical method for 32 designer drugs at low LOQ.

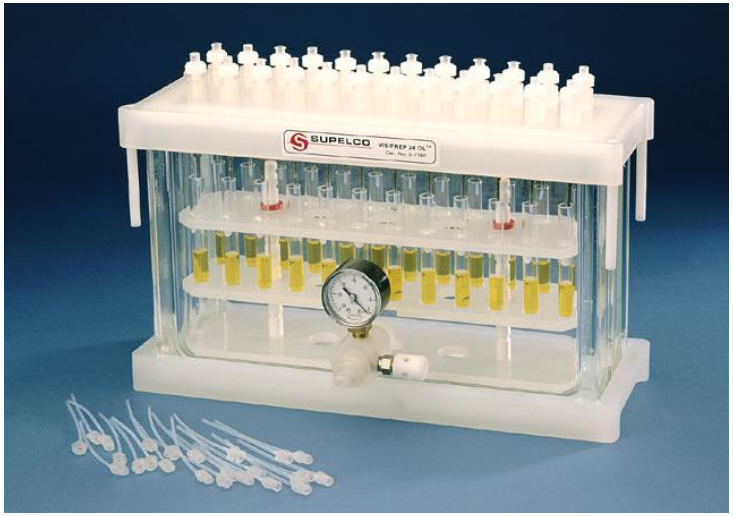
Drug Class	Sub-Class	Basic Structure	Compounds	
Phenethylamines	2,5-dimethoxy-amphetamines		R^1 = Br, R^2 = H R^1 = C_2H_5, R^2 = H R^1 = CH_3, R^2 = H R^1 = O-CH_3, R^2 = H	DOB DOET DOM TMA
	2Cs		R = Br R = C_2H_5 R = I R = S-$CH(CH_3)_2$ R = S-C_3H_7	2C-B 2C-E 2C-I 2C-T-4 2C-T-7
	3,4-methylenedioxyamphetamines		R = H R = CH_3 R = C_2H_5	MDA MDMA MDEA
	Amphetamines		R = H R = CH_3 R = C_2H_5	Amphetamine Methamphetamine Ethylamphetamine
	Pyrrolidinophenones		R^1 = R^2 = O-CH_2-O, R^3 = C_3H_7	MDPV
	β-keto-amphetamines	 	R^1 = R^2 = CH_3 R^1 = R^2 = H R^1 = H, R^2 = CH_3 R^1 = O-CH_3, R^2 = CH_3 R^1 = CH_3, R^2 = C_2H_5 R^1 = F, R^2 = CH_3 R^1 = R^2 = CH_3 R^1 = C_2H_5, R^2 = CH_3	Mephedrone Cathinone Methcathinone Methedrone 4-Methylethcathinone Flephedrone Methylone Butylone
Piperazines	Benzylpiperazines		R = H R = CH_2-C_6H_5	BZP DBZP
	Phenylpiperazines		R = Cl R = CF_3	mCPP TFMPP
Tryptamines			R^1 = R^3 = R^4 = H, R^2 = CH_3 R^1 = R^2 = H, R^3 = R^4 = CH_3 R^1 = O-CH_3, R^2 = H, R^3 = R^4 =CH_3 R^1 = O-CH_3, R^2 = H, R^3 = R^4 =C_3H_7	AMT DMT 5-MeO-DMT 5-MeO-DiPT

Instrumentation

- Agilent 1290 Infinity Binary Pump LC.
- Agilent 6460 triple quadrupole MS/MS.
- Jet Streaming technology, electrospray ionization (ESI).
- Data acquired in Dynamic MRM mode with positive ESI.

Extraction Method

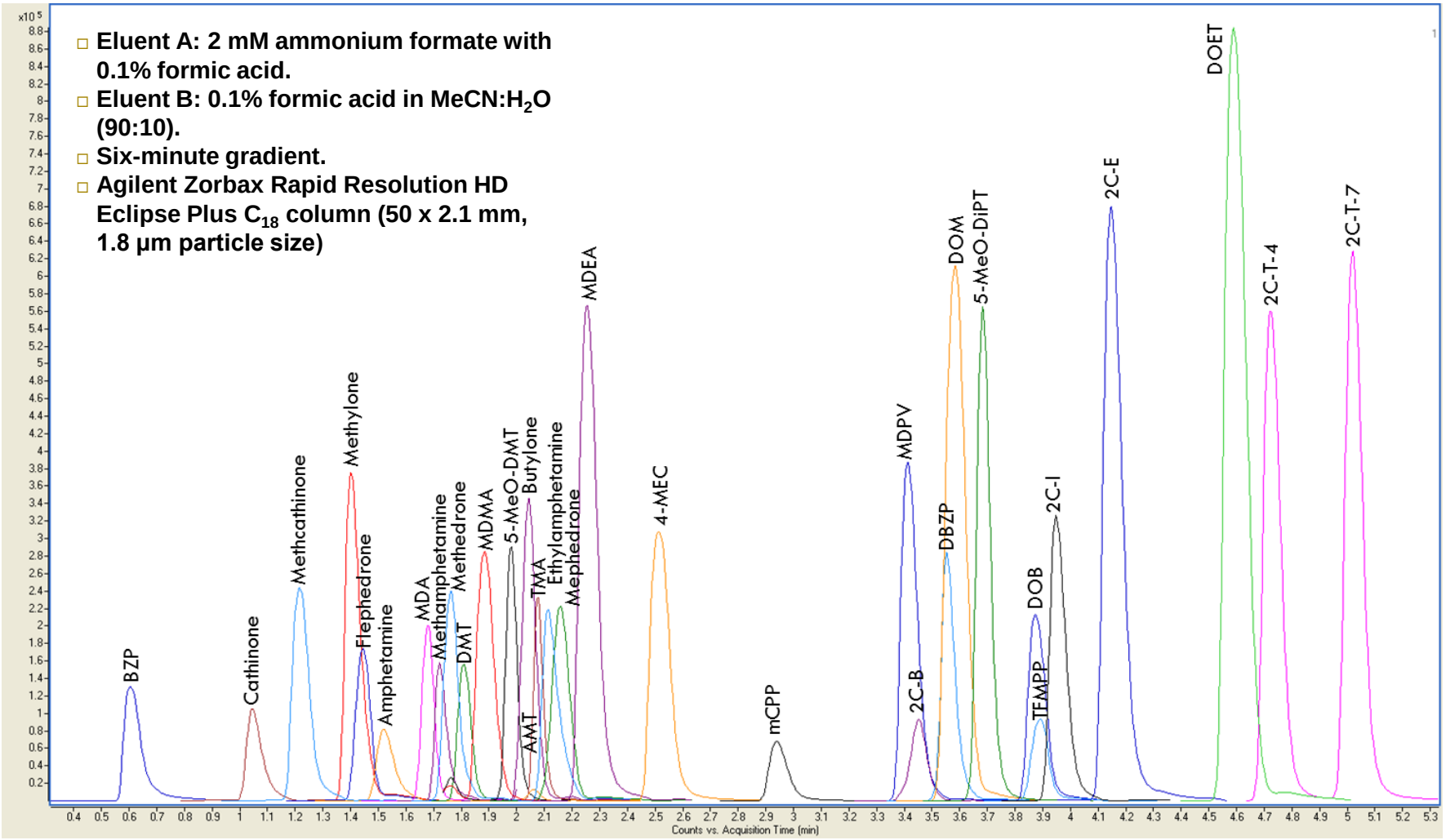
- Extraction from serum or blood.
- Restek Drug Prep I (mixed-mode) SPE cartridge.
- SPE vacuum manifold.



- Dilute 1 mL sample with 2 mL phosphate buffer.
- Condition column with 3 mL MeOH, 3 mL H_2O, and 1 mL buffer.
- Load sample.
- Wash with 1 mL H_2O, 1 mL 0.1 M acetic acid, 1 mL MeOH.
- Elute with 3 mL CH_2Cl_2:IPA:NH_3OH (80:20:2).
- Acidify with HCl:IPA (1:3 to prevent evaporation of volatile compounds).
- Dry down and reconstitute in mobile phase.

Chromatographic Separation

- **Eluent A: 2 mM ammonium formate with 0.1% formic acid.**
- **Eluent B: 0.1% formic acid in MeCN:H_2O (90:10).**
- **Six-minute gradient.**
- **Agilent Zorbax Rapid Resolution HD Eclipse Plus C_{18} column (50 x 2.1 mm, 1.8 µm particle size)**



MS/MS Data

Quantifier

Determined using Agilent Optimizer Software

Retention time used for Dynamic MRM

No.	Drug	Transitions*	CE (V)	Fragmentor (V)	tR (min)	Internal Standard
1	DOB	**274.01 → 256.9**	14	100	3.846 ± 0.00646	d6-Amphetamine
		274.01 → 228.9	10			
2	DOET	**224.3 → 207**	5	85	4.547 ± 0.00230	d6-Amphetamine
		224.3 → 91	49			
3	DOM	**210.3 → 193.1**	5	75	3.538 ± 0.00000	d6-Amphetamine
		210.3 → 165	13			
4	TMA	**226.3 → 209**	5	80	2.075 ± 0.00164	d6-Amphetamine
		226.3 → 91	45			
5	2C-B	**260.01 → 242.9**	4	90	3.403 ± 0.00216	d5-MDMA
		260.01 → 227.9	6			
6	2C-E	**210.3 → 193**	5	80	4.119 ± 0.01371	d5-MDMA
		210.3 → 163	25			
7	2C-I	**308.1 → 290.9**	9	90	3.906 ± 0.00316	d5-MDMA
		308.1 → 91	49			
8	2C-T-4	**256.4 → 239**	5	90	4.675 ± 0.00000	d5-MDMA
		256.4 → 197	17			
9	2C-T-7	**256.4 → 239**	9	85	4.959 ± 0.00491	d5-MDMA
		256.4 → 166.9	29			
10	MDA	**180.1 → 163**	4	70	1.658 ± 0.00536	d6-Amphetamine
		180.1 → 105	20			
11	MDEA	**208.14 → 163**	8	90	2.220 ± 0.00491	d5-MDMA
		208.14 → 105	24			
12	MDMA	**194.1 → 163**	8	85	1.849 ± 0.00491	d5-MDMA
		194.1 → 105	24			
13	Amphetamine	**136.11 → 91**	16	75	1.490 ± 0.00000	d6-Amphetamine
		136.11 → 119	4			
14	Methamphetamine	**150.13 → 91**	16	80	1.715 ± 0.00000	d5-MDMA
		150.13 → 119	4			
15	Ethylamphetamine	**164.11 → 91**	20	85	2.093 ± 0.00219	d5-MDMA
		164.11 → 119	8			
16	MDPV	**276.3 → 126**	25	130	3.383 ± 0.00258	d3-Methylone
		276.3 → 135	25			
17	Mephedrone	**178.25 → 160**	10	85	2.123 ± 0.00151	d3-Mephedrone
		178.25 → 144	30			
18	Cathinone	**150.2 → 132**	10	80	1.031 ± 0.00130	d3-Mephedrone
		150.2 → 117	22			
19	Methcathinone	**164.23 → 146**	10	85	1.196 ± 0.00114	d3-Mephedrone
		164.23 → 130	34			

No.	Drug	Transitions*	CE (V)	Fragmentor (V)	tR (min)	Internal Standard
20	Methedrone	**194.25 → 176**	10	80	1.745 ± 0.00109	d3-Mephedrone
		194.25 → 161	18			
21	4-MEC	**192.28 → 174.1**	10	95	2.482 ± 0.00100	d3-Mephedrone
		192.28 → 145	18			
22	Flephedrone	**182.21 → 164**	10	85	1.422 ± 0.00167	d3-Mephedrone
		182.21 → 148	34			
23	Methylone	**208.24 → 160**	14	80	1.397 ± 0.00083	d3-Methylone
		208.24 → 132	26			
24	Butylone	**222.26 → 174**	14	95	2.035 ± 0.00228	d3-Methylone
		222.26 → 204	10			
25	BZP	**177.11 → 91**	20	100	0.589 ± 0.00526	d7-BZP
		177.11 → 65	50			
26	DBZP	**267.21 → 91**	32	125	3.520 ± 0.00785	d7-BZP
		267.21 → 175	12			
27	mCPP	**197.11 → 153.9**	20	120	2.878 ± 0.00114	d4-TFMPP
		197.11 → 118	36			
28	TFMPP	**231.11 → 188**	20	125	3.826 ± 0.00070	d4-TFMPP
		231.11 → 118	44			
29	AMT	**175.2 → 158**	9	75	2.037 ± 0.00181	d6-Amphetamine
		175.2 → 143	25			
30	DMT	**189.11 → 58.1**	8	85	1.775 ± 0.00249	d5-MDMA
		189.11 → 144	16			
31	5-MeO-DMT	**219.3 → 58.1**	9	85	1.955 ± 0.00151	d5-MDMA
		219.3 → 174	9			
32	5-MeO-DiPT	**275.4 → 174**	17	100	3.627 ± 0.00187	d5-MDMA
		275.4 → 114.1	13			
33	d6-Amphetamine (IS)	**142.25 → 93**	13	75	1.470 ± 0.00194	-
		142.25 → 125.1	5			
34	d5-MDMA (IS)	**199.29 → 165**	9	90	1.839 ± 0.00234	-
		199.29 → 107	25			
35	d3-Mephedrone (IS)	**181.27 → 163**	9	90	2.115 ± 0.00158	-
		181.27 → 148	21			
36	d3-Methylone (IS)	**211.21 → 163**	13	85	1.390 ± 0.00044	-
		211.21 → 135	29			
37	d7-BZP (IS)	**184.11 → 98.1**	21	105	0.562 ± 0.00089	-
		184.11 → 70.1	57			
38	d4-TFMPP (IS)	**235.11 → 190**	21	125	3.815 ± 0.00268	-
		235.11 → 46.1	21			

Validation Parameters

- Selectivity:
  - Screen individual drugs for interferences from other compounds.
  - Screen samples of blank pooled serum.
- Matrix effects, extraction recovery, and process efficiency:
  - Compare concentrations of drugs between neat samples, spiked samples, and the spiked extracts of blank samples.
- Stability:
  - Analyze extracts of samples that are left in the auto-sampler repeatedly for over 30 h.
- Linearity & LOQ:
  - Replicate calibrators analyzed at various concentrations and then analyzed using Agilent Quant software to examine calibration curves.

Main Stage Validation

- Determines precision, accuracy, & freeze-thaw stability over a series of 8 runs
- Acceptance criteria for method requires ±15% bias (±20% around the LLOQ) and <15% R.S.D. for precision (<20% R.S.D. around the LLOQ

25 ppb | 100 ppb | 250 ppb | 10 ppb

Run	Calibration samples (six levels)	Validation samples						
		Low		Medium	High		Optional	LLOQ
		Precision and accuracy	Freeze/thaw stability	Precision and accuracy	Precision and accuracy	Freeze/thaw stability		Precision and accuracy
1	6	2	6	2	2	6		2
2	6	2		2	2			2
3	6	2		2	2			2
4	6	2	6	2	2	6		2
5	6	2		2	2			2
6	6	2		2	2			2
7	6	2		2	2			2
8	6	2		2	2			2

Peters, F. T. Method validation using LC-MS. In *Applications of LC-MS in Toxicology*, Polettini, A., Ed.; Pharmaceutical Press: London, UK, 2006; pp 71-96.

Validation Results

- Selective for targeted analytes.
- Interfering peaks from matrix were negligible; no co-elutions.
- LODs in the range of 10 -100 pg/mL.
- LOQs in the range of 1-10 ng/mL.
- Linear between LOQ and 250 ng/mL.
- Analytes stable for the length of the batch run.
- Precision and accuracy within acceptance limits.
- **Method was fully validated for the analysis of 32 designer drug analytes in serum.**

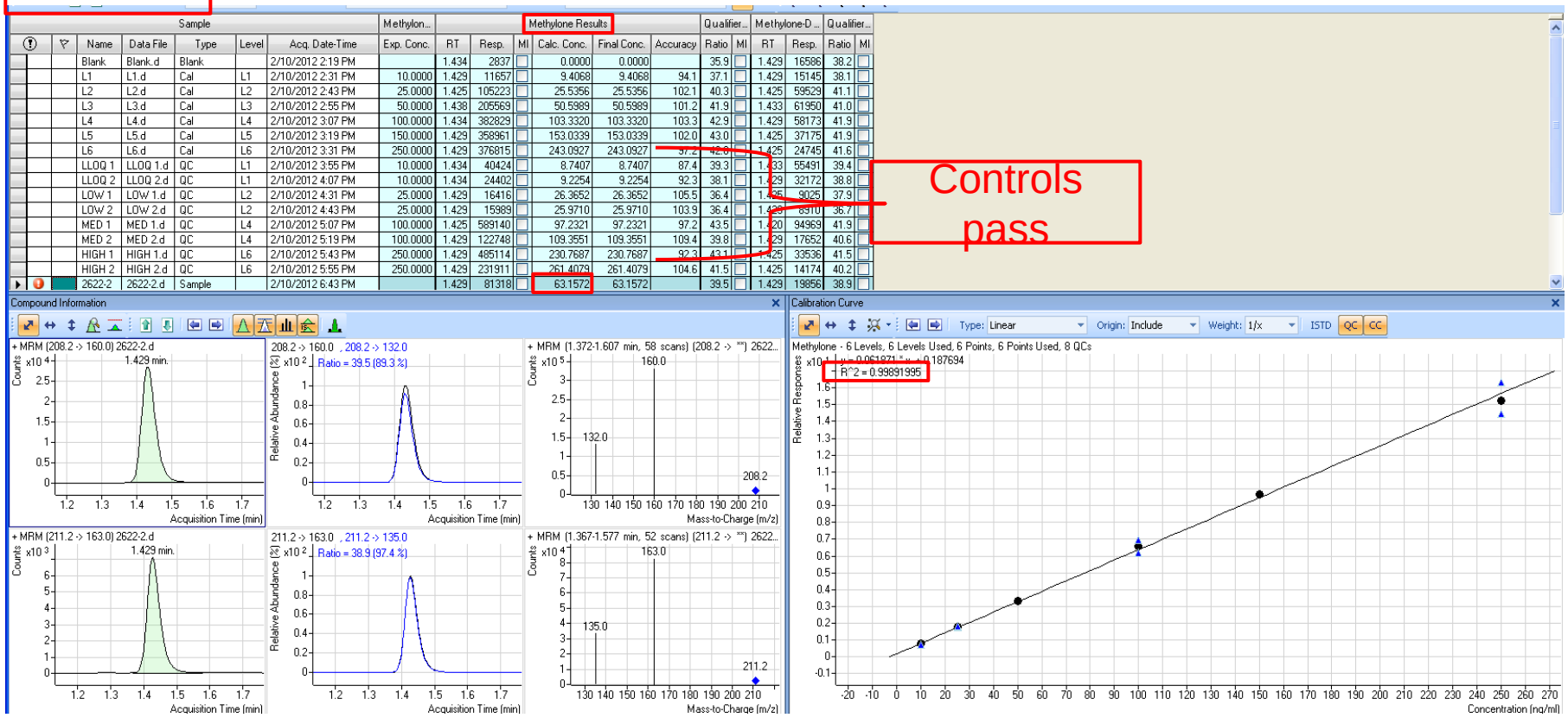
Case Sample Analysis

- PM blood specimens from two decedents - MDME Toxicology Department.
- Suspicion of designer drug involvement.
- Method was able to confirm MDPV, which was missed in initial GC-MS urine screens.
- Other results were consistent with GC-MS screening data.
- Urine immunoassays for amphetamines negative.

Compound	Case 1	Case 2
BZP		>250 ng/mL
Methylone	63 ng/mL	
MDA		36 ng/mL
MDMA	58 ng/mL	115 ng/mL
MDPV		**11 ng/mL**
5-MeO-DiPT		>250 ng/mL
TFMPP		93 ng/mL

Example Results for Case Sample

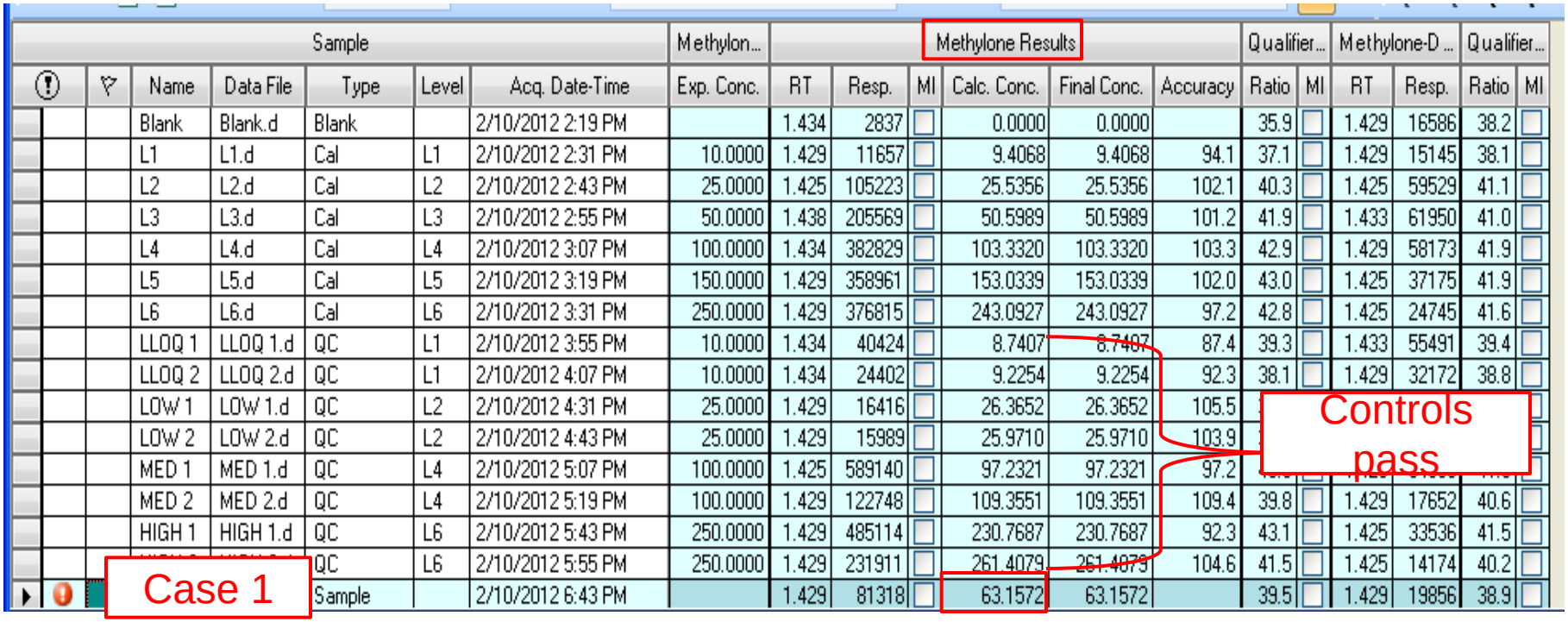
Case 1



Sample						Methylon...	Methylone Results						Qualifier...	Methylone-D...			Qualifier...
		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	RT	Resp.	Ratio	MI
		Blank	Blank.d	Blank		2/10/2012 2:19 PM		1.434	2837		0.0000	0.0000		35.9		1.429	16586	38.2	
		L1	L1.d	Cal	L1	2/10/2012 2:31 PM	10.0000	1.429	11657		9.4068	9.4068	94.1	37.1		1.429	15145	38.1	
		L2	L2.d	Cal	L2	2/10/2012 2:43 PM	25.0000	1.425	105223		25.5356	25.5356	102.1	40.3		1.425	59529	41.1	
		L3	L3.d	Cal	L3	2/10/2012 2:55 PM	50.0000	1.438	205569		50.5989	50.5989	101.2	41.9		1.433	61950	41.0	
		L4	L4.d	Cal	L4	2/10/2012 3:07 PM	100.0000	1.434	382829		103.3320	103.3320	103.3	42.9		1.429	58173	41.9	
		L5	L5.d	Cal	L5	2/10/2012 3:19 PM	150.0000	1.429	358961		153.0339	153.0339	102.0	43.0		1.425	37175	41.9	
		L6	L6.d	Cal	L6	2/10/2012 3:31 PM	250.0000	1.429	376815		243.0927	243.0927	97.2	42.8		1.425	24745	41.6	
		LLOQ 1	LLOQ 1.d	QC	L1	2/10/2012 3:55 PM	10.0000	1.434	40424		8.7407	8.7407	87.4	39.3		1.433	55491	39.4	
		LLOQ 2	LLOQ 2.d	QC	L1	2/10/2012 4:07 PM	10.0000	1.434	24402		9.2254	9.2254	92.3	38.1		1.429	32172	38.8	
		LOW 1	LOW 1.d	QC	L2	2/10/2012 4:31 PM	25.0000	1.429	16416		26.3652	26.3652	105.5	36.4		1.425	9025	37.9	
		LOW 2	LOW 2.d	QC	L2	2/10/2012 4:43 PM	25.0000	1.429	15989		25.9710	25.9710	103.9	36.4		1.429	8910	35.7	
		MED 1	MED 1.d	QC	L4	2/10/2012 5:07 PM	100.0000	1.425	589140		97.2321	97.2321	97.2	43.5		1.420	94969	41.9	
		MED 2	MED 2.d	QC	L4	2/10/2012 5:19 PM	100.0000	1.429	122748		109.3551	109.3551	109.4	39.8		1.429	17652	40.6	
		HIGH 1	HIGH 1.d	QC	L6	2/10/2012 5:43 PM	250.0000	1.429	485114		230.7687	230.7687	92.3	43.1		1.425	33536	41.5	
		HIGH 2	HIGH 2.d	QC	L6	2/10/2012 5:55 PM	250.0000	1.429	231911		261.4079	261.4079	104.6	41.5		1.425	14174	40.2	
		2622-2	2622-2.d	Sample		2/10/2012 6:43 PM		1.429	81318		63.1572	63.1572		39.5		1.429	19856	38.9	

Example Results for Case Sample

Case 1



Sample						Methylon...	Methylone Results						Qualifier...	Methylone-D ...		Qualifier...	
Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	RT	Resp.	Ratio	MI
Blank	Blank.d	Blank		2/10/2012 2:19 PM		1.434	2837		0.0000	0.0000		35.9		1.429	16586	38.2	
L1	L1.d	Cal	L1	2/10/2012 2:31 PM	10.0000	1.429	11657		9.4068	9.4068	94.1	37.1		1.429	15145	38.1	
L2	L2.d	Cal	L2	2/10/2012 2:43 PM	25.0000	1.425	105223		25.5356	25.5356	102.1	40.3		1.425	59529	41.1	
L3	L3.d	Cal	L3	2/10/2012 2:55 PM	50.0000	1.438	205569		50.5989	50.5989	101.2	41.9		1.433	61950	41.0	
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L5	L5.d	Cal	L5	2/10/2012 3:19 PM	150.0000	1.429	358961		153.0339	153.0339	102.0	43.0		1.425	37175	41.9	
L6	L6.d	Cal	L6	2/10/2012 3:31 PM	250.0000	1.429	376815		243.0927	243.0927	97.2	42.8		1.425	24745	41.6	
LLOQ 1	LLOQ 1.d	QC	L1	2/10/2012 3:55 PM	10.0000	1.434	40424		8.7407	8.7407	87.4	39.3		1.433	55491	39.4	
LLOQ 2	LLOQ 2.d	QC	L1	2/10/2012 4:07 PM	10.0000	1.434	24402		9.2254	9.2254	92.3	38.1		1.429	32172	38.8	
LOW 1	LOW 1.d	QC	L2	2/10/2012 4:31 PM	25.0000	1.429	16416		26.3652	26.3652	105.5						
LOW 2	LOW 2.d	QC	L2	2/10/2012 4:43 PM	25.0000	1.429	15989		25.9710	25.9710	103.9						
MED 1	MED 1.d	QC	L4	2/10/2012 5:07 PM	100.0000	1.425	589140		97.2321	97.2321	97.2						
MED 2	MED 2.d	QC	L4	2/10/2012 5:19 PM	100.0000	1.429	122748		109.3551	109.3551	109.4	39.8		1.429	17652	40.6	
HIGH 1	HIGH 1.d	QC	L6	2/10/2012 5:43 PM	250.0000	1.429	485114		230.7687	230.7687	92.3	43.1		1.425	33536	41.5	
		QC	L6	2/10/2012 5:55 PM	250.0000	1.429	231911		261.4079	261.4079	104.6	41.5		1.425	14174	40.2	
		Sample		2/10/2012 6:43 PM		1.429	81318		63.1572	63.1572		39.5		1.429	19856	38.9	

Controls pass

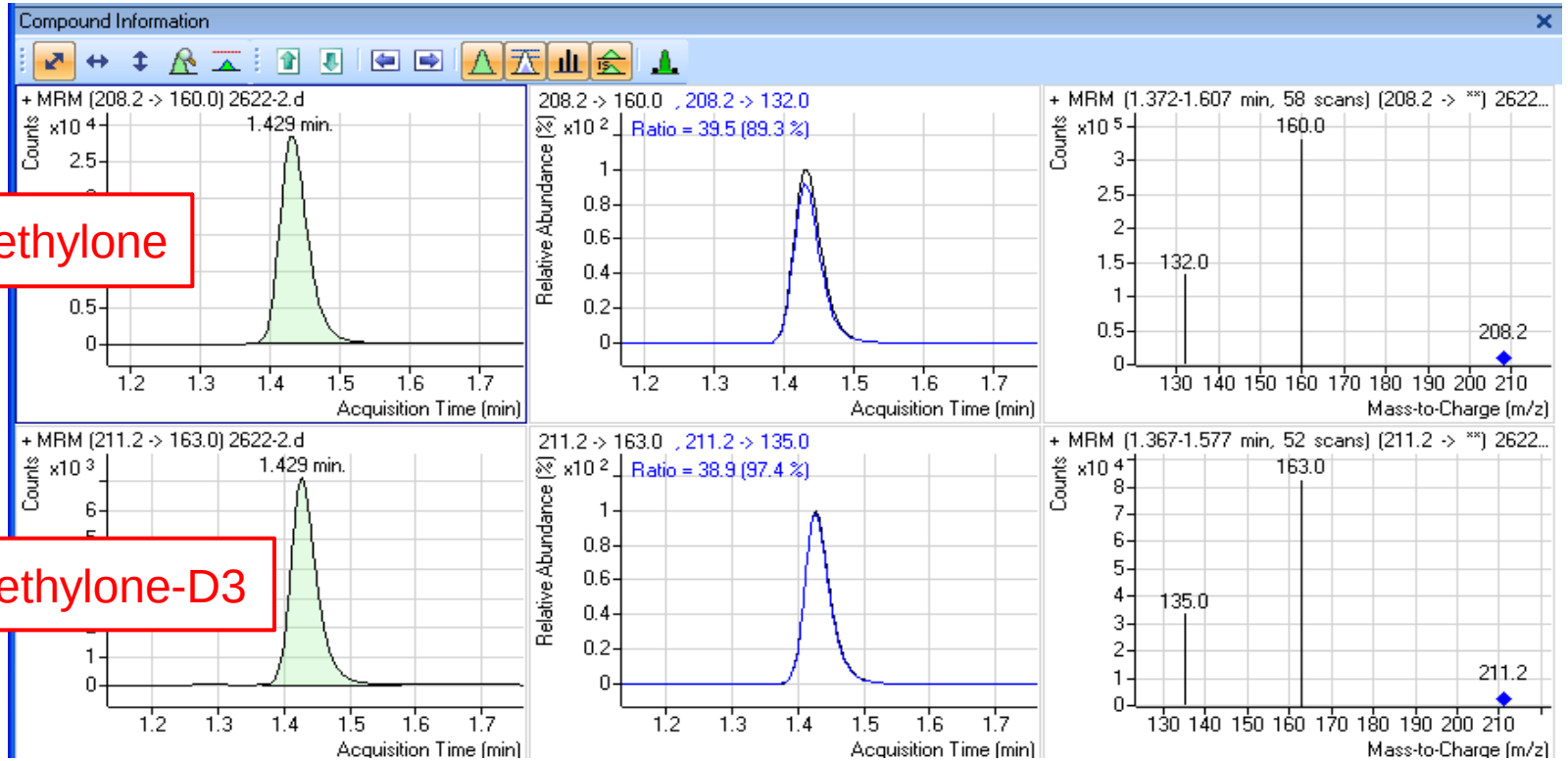
Case 1

Example Results for Case Sample

Case 1

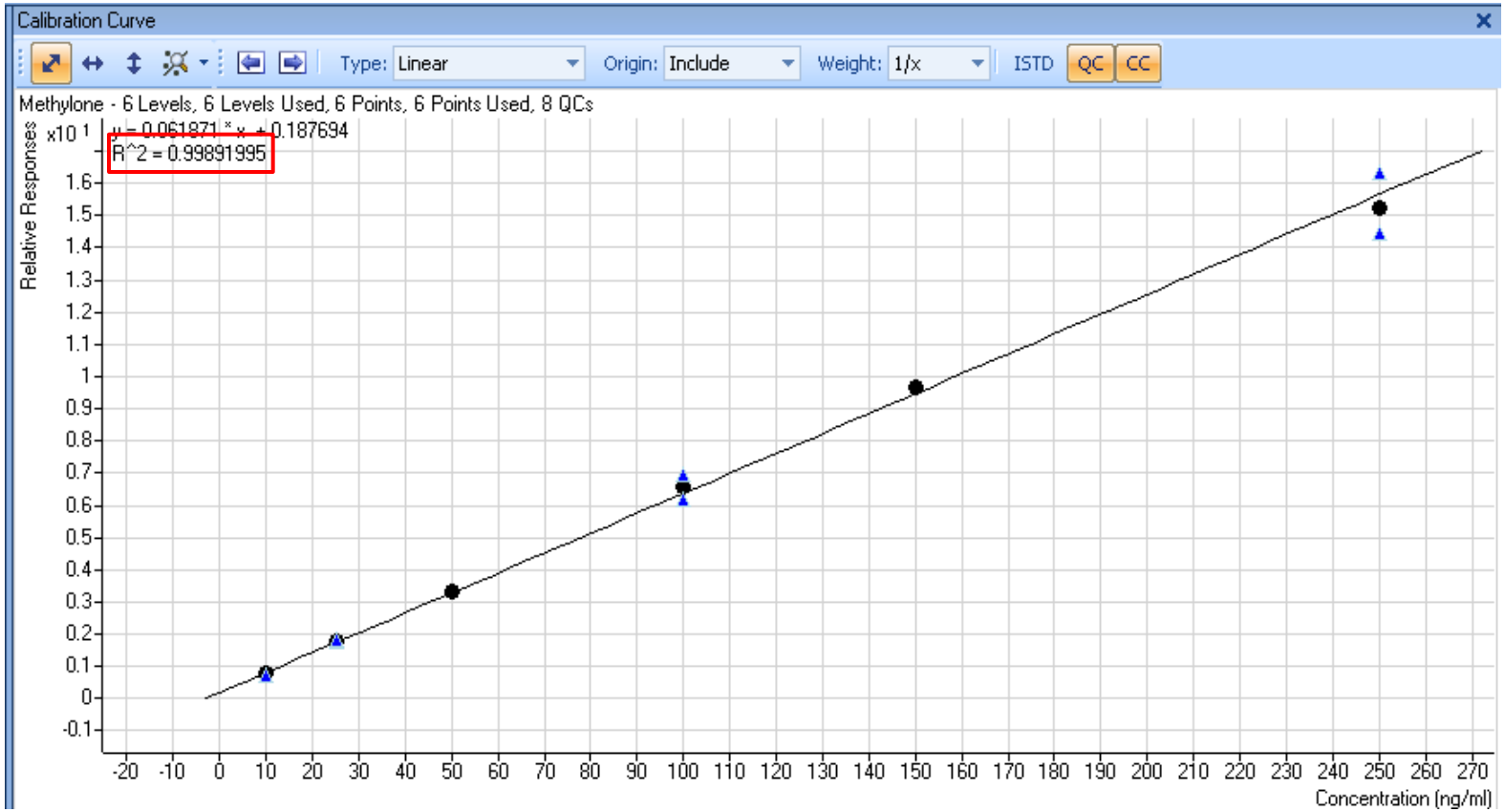
Methylone

Methylone-D3



Example Results for Case Sample

Case 1



Conclusions

- LC-MS/MS method was fully validated according to established international guidelines (as recommended by Peters, et al.) for the confirmation and quantitation of 32 designer drug entities at low LOQ.
- Method was successfully applied to the analysis of two post-mortem samples that were suspected of involving bath salts

Ongoing Work

- Assessment of cross-reactivity of designer drugs in commercial immunoassay screening platforms.
  - Funded by NIJ grant 2011-DN-BX-K559.

- Development of comprehensive LC-QQQ and LC-QTOF MS/MS libraries for designer drugs.
  - Funded by a collaborative agreement and instrument loans from Agilent Technologies, Inc.

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- Agilent Technologies, Inc.
- National Institute of Justice.

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Questions?